SAMPLES PREPARATION

1. PURPOSE AND SCOPE

Several types of samples can be used for the detection of viruses responsible for vesicular diseases: epithelial fragments (mouth ulcer/vesicle wall flaps), vesicular fluid, whole blood, serum, supernatant from infected cells.

Sample/fluid preparation is the preliminary step before conducting the various tests dedicated to the detection of these viruses (detection of the virus, a viral antigen or the viral genome).

2. NORMATIVE REFERENCES

Official analytical method of the World Organisation for Animal Health (OIE), described in Chapter 3.1.8. of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, published in 2018.

3. DEFINITIONS

Not applicable

4. PRINCIPLE OF THE METHOD

Some samples can be used directly, without any preparation. Epithelial flaps have to be homogenised and clarified.

5. EQUIPMENT AND MATERIALS

- Class II microbiological safety cabinet (MSC)
- Refrigerated chamber: +5°C (± 3°C)
- Freezer < -70°C
- Refrigerated centrifuge
- Ribolyser
- Ribolyser tubes
- Sterile mortars and pestles
- Weighing scale (+/- 0.1 g)
- (Sterile) small standard laboratory equipment: Erlenmeyer flasks with caps, beakers, graduated cylinders, magnetic rods, tubes with caps, 1.5 ml microtubes, cryotubes, pipettes, etc.
- Petri dishes
- Single-use tweezers and scalpels

6. REAGENTS AND PRODUCTS

6.1. LIST

- Culture medium (Minimum Essential Medium (MEM) with Earle's salts) (Invitrogen)
- Distilled or deionised water
- Antibiotic mixture (Invitrogen): components of the mixture = Penicillin G Sodium 10,000 units per ml and Streptomycin 10,000 µg per ml
- Sterile sand, a mortar, a pestle
- Homogenisation kit (Svanova)(Optionnal)
- Ribolyser and related tubes (Optional)

6.2. PREPARATION

6.2.1. Preparation of the cell culture medium

It is a cell culture medium supplemented with antibiotics used for the preparation of samples (2% antibiotics) and the recovery of cell monolayers after viral inoculation (1% antibiotics). Store at +5°C (± 3°C) and bring to ambient temperature before using.

6.2.2. Preparation of the sand

Start by sterilising the sand by autoclaving (30-minute cycle at 121°C). Divide into 3 to 5 g aliquots and store at ambient temperature (20°C ± 5°C).

7. PROCEDURE

7.1. EPITHELIA

7.1.1. Washing

Epithelia sent dry: Wash the epithelial fragments in a small amount of MEM (2% antibiotics): take with sterile tweezers and bubble in a small vial filled with this medium.

Epithelia sent in a storage medium containing glycerol: Wash the epithelial fragments at least six times in culture medium (2% antibiotics) (glycerol is toxic to cells).

7.1.2. Weighing

After washing, place the epithelial fragments in a sterile Petri dish. If necessary, use a scalpel and tweezers to cut them into small fragments. Weigh them.

NB: When there is abundant material, do not homogenise all of the mouth-ulcer flaps but rather set some of them aside for future testing. Store these fragments at < -70°C.

7.1.3. Homogenisation

Using a mortar: cut the sample into small fragments and homogenise with a small amount of sterile sand and MEM (2% antibiotics) so as to obtain a 10% weight/vol suspension (if not enough sample, produce a 5% suspension). NB: it is possible to use the Svanova homogenisation kit instead of the mortar and sand.

Transfer the homogenate to a sterile centrifuge tube and centrifuge at 3000 xg, for 10 minutes, at +5°C (± 3°C).

Collect the supernatant in a sterile tube.

Store at +5°C (± 3°C) if used on the same day (otherwise at < -70°C).

Using a ribolyser, cut the sample into small fragments and place 0.1 g (max) in a ribolyser tube. Add 1 ml of MEM (2% antibiotics) so as to obtain a 10% weight/vol suspension (if not enough sample, produce a 5% suspension). Prepare as many tubes as necessary. ***Important: write the name of the sample on the tubes, not on the caps.***

Complete at least two 20-second cycles with the ribolyser. Put the samples on ice between cycles for 1 min.

Centrifuge at 3000 xg for 10 minutes.

Collect and pool the supernatants of the same sample in a sterile tube.

Store at +5°C (± 3°C) if used on the same day (otherwise at < -70°C).

7.2. VESICULAR FLUID

Use directly, as a matrix for virological and molecular analyses. If the quantity is insufficient, test in a 1:10 or 1:5 dilution depending on the quantity. Store at +5°C (± 3°C) if used on the same day (otherwise at < -70°C).

7.3. WHOLE BLOOD WITH ANTICOAGULANT

Use directly, as a matrix for molecular and/or virological analyses.

Store blood at +5°C (± 3°C) if used within 48 hours (otherwise at -20°C ± 5°C).

7.4. SERUM OR BLOOD IN A DRY TUBE

Centrifuge for 10 minutes at 500 xg, then recover the supernatant and use it as a matrix for serological and/or molecular analyses.

For seroneutralisation, heat-inactivate the serum for 30 minutes at 56°C.

Store serum at +5°C (± 3°C) if used within 48 hours (otherwise at -20°C ± 5°C).

7.5. SUPERNATANT FROM INFECTED CELL CULTURES

- Freeze at < -70°C and thaw the plate containing the infected cell cultures
- Recover the viral suspension in a sterile centrifuge tube
- Centrifuge at 3000 xg for 10 minutes, at +5°C (± 3°C).
- Collect the supernatant in a sterile tube.
- This supernatant can be used as a matrix for virological analyses (Ag ELISA, second passage in cell culture) and for molecular analyses (RT-PCR and RT-qPCR).
- Store + 5°C (± 3°C) if used on the same day (otherwise freeze at < -70°C).